

Applicant: Basheer et al.  
Application No.: 10/700,320  
Examiner: D. Carr

### **Amendments to the Claims**

1. (Previously presented) A process for the production of 1,2-diacylated-glycerophospholipids and their synthetic or natural analogues, wherein the 1-acyl and 2-acyl groups are predetermined and identical, comprising contacting in a microaqueous environment a glycerophospholipid with a carboxylic acid acyl donor in the presence of a phospholipase enzyme capable of catalyzing an esterification/transesterification/acylation at both the *sn*-1 and *sn*-2 positions of said glycerophospholipid.
2. (Previously presented) A process according to claim 1, wherein the water content in the reaction mixture is less than 30% by volume of the volume of the reaction mixture, preferably less than 5%, more preferably 0.5-3%.
3. (Previously presented) A process according to claim 1, wherein said glycero-phospholipid is selected from the group consisting of glycerophosphoryl choline (GPC) and derivatives thereof, in which derivatives the choline moiety is replaced by ethanolamine, serine or an alcohol, preferably inositol or glycerol.
4. (Previously presented) A process according to claim 1, wherein said carboxylic acid acyl donor is a fatty acid acyl donor.
5. (Previously presented) A process according to claim 4, wherein said fatty acid acyl donor is a saturated or unsaturated, short-, medium- or long-chained linear or branched fatty acid derivative.
6. (Previously presented) A process according to claim 5, wherein said fatty acid derivative is selected from the group consisting of free fatty acid, fatty acid chloride, fatty acid alkyl ester, fatty acid vinyl ester and fatty acid anhydride.

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7. (Previously presented) A process according to claim 1, wherein the phospholipase enzyme is optionally immobilized on an insoluble matrix and is optionally surfactant-coated.
8. (Previously presented) A process according to claim 1, wherein said phospholipase enzyme is immobilized on an insoluble matrix and is optionally surfactant-coated.
9. (Previously presented) A process according to claim 8, wherein said immobilized phospholipase is surfactant-coated.
10. (Previously presented) A process according to claim 1, wherein the phospholipase enzyme that catalyzes the acylation at both the *sn*-1 and *sn*-2 positions of said glycerophospholipid is derived from the genus *Aspergillus*.
11. (Previously presented) A process according to claim 10, wherein said *Aspergillus* is SANK 11870.
12. (Previously presented) A process according to claim 1, wherein the conversion yield of said glycerophospholipid to 1,2-diacyl-glycero-phospholipid is at least 20%.
13. (Previously presented) A process according to claim 1, wherein said glycerophospholipid is glycerophosphatidylcholine (GPC).
14. (Previously presented) A process for the production of 1,2-diacylated-glycerophospholipids, and their synthetic or natural analogues, in which the 1-and 2-acyl groups are identical and are predetermined, comprising contacting in a microaqueous environment a glycerophospholipid with a carboxylic acid acyl donor, preferably a fatty acid derivative capable of providing the same desired acyl group in the presence of a phospholipase enzyme capable of catalyzing an acylation at both the *sn*-1 and *sn*-2 positions of said glycerophospholipid.

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15. (Previously presented) A process according to claim 1, wherein 1-monoacyl-2-lyso-glycerophospholipid is formed when a solvent, preferably an organic solvent is used.
16. (Previously presented) A process according to claim 15, wherein said solvent is *tert*-butanol.
17. (Previously presented) A process according to claim 1, wherein the product is any one of 1,2-di-acyl-glycerophosphatidylcholine and analogue thereof.
18. (Previously presented) A process according to claim 17, wherein the product is 1,2-dilauroyl-glycerophosphatidylcholine.
19. (Previously presented) A process according to claim 17, wherein the product is 1,2-dimyristiyl-glycerophosphatidylcholine.
20. (Previously presented) A process according to claim 17, wherein the product is 1,2-dipalmitiyl-glycerophosphatidylcholine.
21. (Previously presented) A process according to claim 18, wherein the glycerophospholipid is glycerophosphatidylcholine and the acyl donor is vinyl laurate.
22. (Previously presented) A process according to claim 19, wherein the glycerophospholipid is glycerophosphatidylcholine and the acyl donor is vinyl myristate.
23. (Previously presented) A process according to claim 19, wherein the glycerophospholipid is glycerophosphatidylcholine and the acyl donor is vinyl palmitate.
24. (Previously presented) A process according to any of claim 7, wherein said optionally

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surfactant-coated phospholipase, capable of catalyzing acylation at *sn*-1 and *sn*-2 positions, is physically, ionically or covalently bound to said insoluble matrix.

25. (Previously presented) A process according to claim 24 wherein said insoluble matrix is selected from the group consisting of adsorbents, ion-exchange resins and activated insoluble matrices.

26. (Previously presented) A process according to claim 24, wherein the insoluble matrix is selected from the group consisting of Dowex<sup>®</sup> 22, Dowex<sup>®</sup> 1x2-40, Dowex, 2x8-100, cellulose phosphate, Amberlite<sup>®</sup> IRA-95, Amberlite<sup>®</sup> IRA-200 Amberlite<sup>®</sup> IRA-900, Amberlite<sup>®</sup> XAD-7, Amberlite<sup>®</sup> XAD-16, Diannon<sup>®</sup> SA-10A, Ectoela<sup>®</sup> cellulose, Sephadex<sup>®</sup> and sulfoxyethylcellulose, Celite, alumina, silica gel, calcium carbonate, aluminum stearate, charcoal and calcium sulfate.

27. (Previously presented) A process according to claim 7, wherein said surfactant is selected from the group consisting of sugar fatty acid esters, sugar alkyl esters, polyol fatty acid esters and polyol alkyl ethers.

28. (Previously presented) A process according to claim 25, wherein the surfactant is sorbitan monolaurate, sorbitan monomyristate, sorbitan monopalmitate and sorbitan monostearate.

29. -31. (Cancelled)

32. (Previously presented) 1-Stearoyl-2-lyso-glycerophospholipids prepared by the process of claim 15.

33. (Cancelled)

34. (Previously presented) A process for the production of 1-acylated-2-lyso-

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glycerophospholipids and their synthetic or natural analogues, wherein the 1-acyl group is predetermined, comprising contacting in a microaqueous environment a glycerophospholipid with a carboxylic acid acyl donor in the presence of a phospholipase capable of catalyzing an acylation at the *sn*-1 position (site) of said glycerophospholipid, in the presence of an organic solvent.

35. (Previously presented) A process according to claim 34, wherein the water content in the reaction mixture is less than 30% by volume of the volume of the reaction mixture, preferably less than 5%, more preferably 0.5-3%.

36. (Previously presented) A process according to claim 34, wherein said carboxylic acid acyl donor is a fatty acid acyl donor.

37. (Previously presented) A process according to claim 36 wherein said fatty acid acyl donor is a saturated or unsaturated, short-, medium- or long-chained linear or branched fatty acid derivative.

38. (Previously presented) A process according to claim 37, wherein said fatty acid derivative is selected from the group consisting of free fatty acid, fatty acid chloride, fatty acid alkyl ester, fatty acid vinyl ester and fatty acid anhydride.

39. (Previously presented) A process according to claim 34, wherein the phospholipase is phospholipase A<sub>1</sub> optionally immobilized on an insoluble matrix and is optionally surfactant-coated.

40. (Previously presented) A process according to claim 34, wherein said phospholipase enzyme is immobilized on an insoluble matrix and is optionally surfactant-coated.

41. (Previously presented) A process according to claim 34, wherein said immobilized

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phospholipasc is surfactant-coated.

42.-43. (Cancelled)